



United States Department of the Interior
U. S. GEOLOGICAL SURVEY
Columbia Environmental Research Center
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Date: January 10, 2013

To: Ed Hammer, USEPA Region 5, Chicago, IL

From: Chris Ingersoll and Ed Little

Subject: USGS Columbia Environmental Research Center (USGS-Columbia) quarterly project summary for the project entitled: "Protectiveness of water or sediment quality guidelines to species of special concern"

Attached please find the 10/01/12 to 12/31/12 quarterly project summary for USGS-Columbia project entitled: "Protectiveness of water or sediment quality guidelines to species of special concern." Please contact us if you have any questions concerning the attached summary (573/876-1819, fax -1896, email cingersoll@usgs.gov, elittle@usgs.gov). Please let us know if there are other individuals that should be receiving our quarterly summaries.

This quarterly summary provides progress on the following 5 tasks associated with the project:

- A. Task 1. Determining the acute and chronic toxicity of ammonia, nitrate and nitrite to amphibians (USGS lead investigator: Ed Little)
- B. Task 2. Developing and demonstrating a sediment toxicity test method with freshwater mussels for assessing sediment contaminants in the Great Lakes Basin and within North America (USGS lead investigator: Chris Ingersoll)
- C. Task 3. Evaluating of conditions used to culture or conduct toxicity tests with the amphipod *Hyaella azteca* (USGS lead investigator: Chris Ingersoll)
- D. Task 4. Assessing the toxicity of sulfate in water to early life stages of fish, snails, and mussels (USGS lead investigator: Ning Wang)
- E. Task 5. Improving concordance of sediment chemistry and toxicity (technical assistance to the USEPA Great Lakes National Program Office; USGS lead investigator: Chris Ingersoll)
- F. Task 6. Water-only toxicity testing of snails and mussels (USGS lead investigator: Ning Wang)
- G. Task 7. Major ion toxicity testing with select toxicity organisms (A: Influence of water hardness on the chronic toxicity of sulfate and chloride to fathead minnows (*Pimephales promelas*) and cladocerans (*Ceriodaphnia dubia*) and B: Acute and chronic toxicity of potassium to select aquatic organisms) USGS lead investigators: Ning Wang and Chris Ingersoll)
- H. Task 8. Water-only toxicity testing with sculpin and darters (USGS lead investigator: John Besser; Task not funded by EPA, but being conducted by USGS support in ongoing

GLRI studies)

cc: Ning Wang, Nile Kemble, John Besser, Bethany Williams, Carl Orazio, Rip Shively, Norman Grannemann (USGS)
Task 1: Chuck Stephan
Task 2 and 6: Chris Barnhart, Missouri State University
Task 3: Dave Mount, Russ Hockett, Chuck Stephan (USEPA), Lisa Taylor, Warren Norwood (Environment Canada), Dave Soucek (INHS)
Task 4, 7, 8: Chuck Stephan (USEPA), Dave Soucek (INHS), Mike Coffee (USFWS)
Task 5: Scott Ireland, Dave Mount (USEPA)

COLUMBIA ENVIRONMENTAL RESEARCH CENTER
UNITED STATES GEOLOGICAL SURVEY, COLUMBIA, MO
QUARTERLY PROGRESS SUMMARY

USGS Basis+ Program Element 42100, USGS Basis+ Project 2145E52 (USGS template/task 240 GLNPO sediment; USGS template/task 251 Hyalella; USGS template/task 252 Sulfate; USGS template/task 253 Mussel sediment; USGS template/task 254: Amphibians; USGS template/task 147 Major ion toxicity)

USGS Project Managers: Chris Ingersoll and Ed Little

Title: Protectiveness of water or sediment quality guidelines to species of special concern

USGS-Columbia is developing quarterly summaries for the USEPA that are intended to describe the status of activities associated with the seven following tasks associated with collaborative research between USGS and USEPA to evaluate the protectiveness of water or sediment quality guidelines to species of special concern (Interagency agreement between USEPA and USGS dated February 1, 2010).

- A. Task 1. Determining the acute and chronic toxicity of ammonia, nitrate and nitrite to amphibians (USGS lead investigator: Ed Little)
- B. Task 2. Developing and demonstrating a sediment toxicity test method with freshwater mussels for assessing sediment contaminants in the Great Lakes Basin and within North America (USGS lead investigator: Chris Ingersoll)
- C. Task 3. Evaluating of conditions used to culture or conduct toxicity tests with the amphipod *Hyalella azteca* (USGS lead investigator: Chris Ingersoll)
- D. Task 4. Assessing the toxicity of sulfate in water to early life stages of fish, and mussels (USGS lead investigator: Ning Wang)
- E. Task 5. Improving concordance of sediment chemistry and toxicity (technical assistance to the USEPA Great Lakes National Program Office; USGS lead investigator: Chris Ingersoll)
- F. Task 6. Water-only toxicity testing of snails and mussels (USGS lead investigator: Ning Wang)
- G. Task 7. Major ion toxicity testing with select aquatic organisms (A: Influence of water

hardness on the chronic toxicity of sulfate or chloride to fathead minnows (*Pimephales promelas*) and cladocerans (*Ceriodaphnia dubia*) and B: Acute and chronic toxicity of potassium or calcium to select aquatic organisms) USGS lead investigators: Ning Wang and Chris Ingersoll)

- H. Task 8. Water-only toxicity testing with sculpin and darters (USGS lead investigator: John Besser; Task not funded by EPA, but being conducted by USGS support in ongoing GLRI studies)

1. What work was accomplished for this past quarter (10/01/12 to 12/31/12)?

- A. Task 1. Determining the acute and chronic toxicity of ammonia, nitrate and nitrite to amphibians
1. Completed acute (96-hour) exposures to chloride (as NaCl) with both southern two-lined salamanders (*Eurycea cirrigera*) and mudpuppies (*Necturus maculosus*), indicating that later-stage larvae of these species can successfully be tested under static renewal conditions. Began data summary and analysis for these tests.
 2. Requested ~1000 mudpuppy larvae from Steve Redman of the Upper Midwest Environmental Sciences Center (La Crosse, WI) for testing in 2013.
 3. Scheduled conference call (1/15/13) to evaluate potential sites for amphibian tests associated with NAWQA Cycle III Regional Synoptic Study.
- B. Task 2. Developing and demonstrating a sediment toxicity test method with freshwater mussels for assessing sediment contaminants in the Great Lakes Basin and within North America
1. USGS personnel participated in scheduled conference calls with USEPA and other interested groups to discuss the status of research conducted and planned associated with this task.
 2. Delayed the start of a sediment toxicity with fatmucket, midge, amphipods and exposed to dilution(s) of a highly contaminated sediment collected under the direction of Scott Ireland from the East Branch of the Grand Calumet River (Task 5). The reason for this delay was that preliminary studies were conducted during the last quarter to refine methods for obtaining known-age midge and amphipods for use in starting these subsequent sediment toxicity exposures. The goal of this next study will be to compare responses of various life stages of amphipods or midge (e.g., 4- vs 7-day old organisms) measuring survival, weight, biomass, or reproduction for amphipods or survival, weight, biomass, or emergence of midge) to the responses of mussels (e.g., 28-d survival, weight, biomass).
 3. No additional exposures were conducted in the mussel sediment exposures because there were no species of the desired age or size available for testing during the past quarter.
- C. Task 3. Evaluating of conditions used to culture or conduct toxicity tests with the amphipod *Hyaella azteca*
1. USGS personnel participated in scheduled conference calls with USEPA and other interested groups to discuss the status of research conducted and planned associated with this task and planned revisions to USEPA and ASTM methods based on the findings from these studies.
 2. Refined USGS methods for culturing known-age amphipods and midge (e.g., to start exposures with 4-d-old vs 7-d-old amphipods or midge).

3. A study was designed to evaluate the influence of age or feeding on amphipods or midge in chronic water or sediment exposures with control water or with control sediment. It is anticipated that this study will be conducted during this next quarter.
- D. Task 4. Assessing the toxicity of sulfate in water to early life stages of fish, snails, and mussels
1. See Task 7.
- E. Task 5. Improving concordance of sediment chemistry and toxicity (technical assistance to the USEPA Great Lakes National Program Office)
1. USGS personnel participated in scheduled conference calls with USEPA and other interested groups to discuss the status of research conducted and planned associated with this task and planned revisions to USEPA and ASTM methods based on the findings from these studies.
 2. Developed a plan for evaluating the response of different life stages of amphipods or midge (e.g., <24-h, 4-d vs 7-day old organisms) and mussels with exposure to dilutions of a highly contaminated and toxic sediment sample collected from the East Branch of the Grand Calumet River. The objective of this next study will be to determine if exposures started with 4-d-old midge improves control performance or if exposures started with 4-d-old amphipods will result in a delay of reproduction past Day 28 of a sediment exposure. Improved feeding methods in water exposures started with about 7-d-old amphipods has resulted in improved growth and reproduction starting before Day 28. Hence, for sediment exposures with amphipods where reproduction is to be determined, it may be desirable to start with younger amphipods (4-d-old rather than 7-d-old) to delay the onset of reproduction past the end of the 28-d sediment exposure. The plan is to start this exposure after completion of the study to evaluate starting age and feeding in control water or in control sediment(s) exposures conducted with amphipods and midge (see Task 3).
 3. Methods for known-age culture of amphipods and midge were evaluated during the past quarter with success (e.g., good survival and growth during 7 to 10 days of culture). Midge isolated on Day 4 tolerated handling well (e.g., transfer into water for 24 hours with good survival or transfer into sand or sediment substrate for 14 days with good survival and growth).
- F. Task 6. Water-only toxicity testing of snails and mussels (USGS lead investigator: Ning Wang)
1. Completed toxicity tests with three mussel species (white heelsplitter, threeridge, and washboard) with the ten Tier 1 chemicals. The preliminary results were summarized in a poster presented in an annual meeting of the Society of Environmental Toxicology and Chemistry in Long Beach, CA, on November 15, 2012 (a copy of the poster is attached).
 2. Conducted multiple range finding tests with fatmucket and ten Tier 2 chemicals. The preliminary data indicate that some organic chemicals, including azoxystrobin, carbaryl, and bifenthrin, decreased substantially over the 4-day static exposures, and aluminum concentrations could not be maintained in our test water with pH about 8.3 (some of the exposure concentrations were above solubility of aluminum at a pH of about 8.3). We plan to continue to work with chemists and others who have used these chemicals in their toxicity tests, to find the methods to maintain more constant exposure concentrations for these chemicals, such as, preparing stock of organic

- chemicals in solvent, “equilibrating” test chambers with the test material before starting the test to minimize the sorption of organic chemicals to the surface of test chambers, and conducting aluminum toxicity test with water at pH 6.
- G. Task 7. Major ion toxicity to select aquatic organisms (USGS lead investigators: Ning Wang and Chris Ingersoll).
1. Task 7a: Sulfate or chloride toxicity influenced by water quality
 - a. Completed a short-term 14-day static-renewal toxicity test with fathead minnows starting with newly fertilized eggs in three test water (CERC 100 mg/L hard water, the 100 hard water with addition of chloride to 25 mg/L, and ASTM moderately hard reconstituted water).
 - b. The preliminary results of the FHM sulfate toxicity test indicated:
 1. FHM in the 100 hard water responded to sulfate toxicity similarly as FHM in the same water we tested in 2011.
 2. Additional chloride in the 100 hard water (10 vs 25 mg Cl/) did not influence sulfate toxicity to FHM.
 3. The effect concentrations of sulfate in the ASTM moderately hard water were greater than those in 100 hard diluted well water (with or without addition of chloride) by a factor of about 2.
 4. Effect concentrations based on biomass were about equal to or even greater than those for survival (biomass endpoint was not more sensitive than survival in this short-term 14-d test, which is contrast to findings in our 2011 34-day sulfate study).The preliminary results were discussed in a conference call with the USEPA Region 5 in December 3, 2012.
 - c. Started a 12-week NaCl exposure of fatmucket. Treatments included 1.0, 0.5, 0.25, 0.13, and 0.063 g NaCl/L with or without the presence of a thin sand substrate. The sand treatment was included to see if presence of an inert substrate might improve performance of the mussels in long-term water toxicity tests. Replicate beakers were destructively sampled on Day 28 of this 84 day exposure to determine survival, weight, and biomass of the mussels. Mean control survival ranged 98 to 100% in both the water-only and sand treatments. While 28-day survival (ranging 83 to 98%) was not significantly reduced with exposure to the highest concentration of NaCl, growth (shell length) was visually observed to be reduced in the highest concentration of NaCl tested.
 - d. Developed a draft memo for EPA Region 5 summarizing the results of 96-h NaCl toxicity tests conduct at our facility over the past several years. The memo will be provided to EPA early in this next quarter.
 2. Task 7b: Potassium or calcium toxicity influenced by water quality
 - a. 96-h KCl toxicity tests were conducted with another two mussels (white heelspliter and washboard).
- H. Task 8. Water-only toxicity testing with sculpin and darters (USGS lead investigator: John Besser)
1. No additional toxicity testing was conducted with sculpin or darters during the past quarter.

2. What problems (or sources of error) were encountered, if any?

None

3. If a problem was encountered, what action was taken to correct it?

Not applicable

4. What work is projected for the new quarterly activity?

A. Task 1. Determining the acute and chronic toxicity of ammonia, nitrate and nitrite to amphibians

1. Continue summarizing data from wood frog and gray tree frog embryos exposed to NaCl, KCl, CaCl₂, and Na₂SO₄ in 2012 tests.
2. Participate in conference calls with USEPA to plan potential test designs for 2013 definitive tests with southern two-lined salamander larvae (*Eurycea cirrigera*) and/or mudpuppies (*Necturus maculosus*). Discuss the possibilities for testing earlier-stage larvae to evaluate life-stage-specific differences in sensitivity seen in previous tests with gray treefrogs (*Hyla versicolor*) and wood frogs (*Lithobates sylvaticus*).
3. Continue discussions about study design for atrazine/nutrient exposures of southern leopard frogs (*Lithobates sphenoccephalus*) or gray treefrogs (*Hyla versicolor*) in association with NAWQA Cycle III Regional Synoptic Study.

B. Task 2. Developing and demonstrating a sediment toxicity test method with freshwater mussels for assessing sediment contaminants in the Great Lakes Basin and within North America

1. Continue evaluating behavior of various species of early life stages of mussels in various sediments using the exposure system described in the October 2012 quarterly summary.
2. Identify additional sediments for evaluating mussel toxicity testing methods (e.g., sediment dilution study with East Branch Grand Calumet River sediment).
3. Continue to develop plans for coordinating sediment testing of mussels as part of the USGS NAWQA agriculture project planned for the Midwest in 2013. This USGS NAWQA project will include sediment toxicity tests conducted with *H. azteca* or *C. dilutus* (information was provided to USEPA in an email dated September 28, 2012). The plan would be to test mussels in about 50 of the 100 sediments to be evaluated in 2013 by the USGS NAWQA program.

C. Task 3. Evaluating of conditions used to culture or conduct toxicity tests with the amphipod *Hyaella azteca*

1. Continue summarizing data for the four 42-d water-only test to evaluate the influence bromide or iodide on the response of *H. azteca* in reconstituted waters.
2. Continue developing plan with the HAAG for conducting inter-laboratory testing of the water-only *H. azteca* toxicity testing method.
3. Continue evaluating methods for culturing and testing known-age amphipod and midge (e.g., <24-h old, 4-d old, 7-d old).
4. Conduct study to evaluate starting age of *H. azteca* or *C. dilutus* in control water or in control sediment using different diets.

- D. Task 4. Assessing the toxicity of sulfate in water to early life stages of fish, snails, and mussels
 - 1. See Task 7.
- E. Task 5. Improving concordance of sediment chemistry and toxicity
 - 1. Continue discussions with USEPA GLNPO regarding ongoing and future options for technical assistance associated with this task (e.g., additional sediments, comparisons of diets for *H. azteca* or *C. dilutus*, evaluate starting age of *H. azteca* in water or in sediment toxicity test [e.g., starting exposures with <24-h old or 4-d-old organisms, rather than 7-day old organisms]).
 - 2. Continue discussions with USEPA and Environment Canada on planned revisions to USEPA and ASTM standard methods for conducting sediment or water toxicity tests with *H. azteca* or *C. dilutus*.
 - 3. Plan for study to conduct additional testing of diluted toxic sediment to evaluate starting age or diet on responses of amphipods or midge in chronic sediment exposures.
- F. Task 6. Water-only toxicity testing of snails and mussels
 - 1. Begin Tier 1 toxicity testing with two commonly test organisms (*Ceriodaphnia dubia*, *Hyalella azteca*) and with snails (*Lymnaea stagnalis*, *Fluminicola* sp., or *Physa gyrina*) cultured in the CERC.
 - 2. We plan to work with chemists and others who have used the Tier 2 chemicals in their toxicity tests, to find the methods to maintain more constant exposure concentrations for some of these chemicals, such as, preparing stock of organic chemicals in solvent, “equilibrating” test chambers with the test material before starting the test to minimize the sorption of organic chemicals to the surface of test chambers, and conducting aluminum toxicity test with water at pH 6.
 - 3. Continue compiling toxicity databases and associated toxicity thresholds for Tier 1 and Tier 2 chemicals (e.g., final acute values).
- G. Task 7. Major ion toxicity to select aquatic organisms (Task 7a: and Task 7b:)
 - 1. Task 7a: Sulfate or chloride toxicity influenced by water quality
 - a. Continuously discuss with the USEPA Region 5 and Dave Mount (USEPA) and Dave Soucek (Illinois Natural History Survey) to design short-term sulfate toxicity test with fathead minnows and other organisms, such as *C. dubia* and mussels, in waters with different Ca:Mg or Na:K ratios.
 - b. Measure shell length and dry weight of surviving mussels in the 28-day NaCl tests with fatmucket, and continue and complete 12-week NaCl exposure with fatmucket.
 - c. Continue discussions with Dave Mount and Dave Soucek on what water quality variables we might want to evaluate in additional NaCl exposures.
 - d. Continue compiling toxicity databases and associated toxicity thresholds (e.g., final acute values) for sulfate and chloride.
 - e. Conduct 24-hour NaCl toxicity test with fatmucket glochidia.
 - f. Finalize memo to be provided to EPA summarizing our historic NaCl 96-h toxicity data for juvenile mussels.
 - 2. Task 7b: Potassium or calcium toxicity influenced by water quality
 - a. Summarize 96-h KCl and CaCl₂ toxicity data for the study conducted with newly transformed juvenile mussels.

- b. Continue discussions with Dave Mount and Dave Soucek on what water quality variables we might want to evaluate in additional KCl or CaCl₂ exposures.
- c. Continue compiling toxicity databases and associated toxicity thresholds (e.g., final acute values) for KCl and for calcium.

H. Task 8. Water-only toxicity testing with sculpin and darters

- 1. No toxicity testing of sculpin or darters are planned for the next quarter.

5. Is the project work on schedule?

For the quarter? Yes

For the project? Yes

6. Does the project funding rate support the work progress?

Yes, given the revised budget period for Task 7 will continue to through June 2016.

7. What has been spent to date?

If requested, USGS will provide USEPA (or other interested groups) summaries of expenditures on the project.

8. Have you submitted a quarterly voucher for reimbursement?

USGS will provide vouchers as necessary.

9. Is there a change in principal investigator?

No

10. If you have a multi-year project with budget periods, have you submitted your request for a funding amendment?

Not applicable